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GENERAL NOTES

Caudal Courtship Glands in the Cave Salamander, *Eurycea lucifuga* (Caudata: Plethodontidae)

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Noble (1929) first reported courtship glands in the tail base of a plethodontid salamander, *Eurycea bislineata.* To date, these sexually dimorphic caudal glands have been demonstrated histologically only for *Desmognathus* (Noble, 1931), Eurycea (Noble, 1929; Sever, 1989; Trauth et al., 1993), and *Plethodon cinereus* (see Houck and Sever, 1994). The term "hedonic gland" was used in previous research (Gadow, 1887; Noble, 1927, 1929, 1931; Rogoff, 1927) to describe a cluster of glands that produced courtship pheromones. Because there was no evidence that the hedonic glands were indeed pleasure giving as the term implied, Arnold (1977) suggested the use of the term "courtship gland." Houck and Sever (1994) adopted the term courtship gland, and we follow their usage when referring to male sexually dimorphic glands within the skin of the tail base.

Caudal courtship glands are located on the dorsal base of the male's tail and hypertrophy during the breeding season; they presumably deliver secretions directly to the female during courtship (Sever, 1989). During the "tail straddling" walk females place their snouts on the male's rump directly over the caudal glands (Arnold, 1977). Caudal courtship pheromones presumably increase female receptivity, thus making her more likely to become inseminated by that male (Houck and Sever, 1994).

Little is known about the reproductive biology of the cave salamander (*Eurycea lucifuga*) in Arkansas. Trauth et al. (1990) reported that females undergo vitellogenesis from February to August; however, no investigation on the breeding cycle and courtship activity of *Eurycea lucifuga* in Arkansas animals has, thus far, been published. In the following paper, we provide the first histological description of caudal courtship glands of the male cave salamander, *Eurycea lucifuga.* Our specific objectives were to: 1) document the structure of caudal courtship glands using light microscopy and 2) compare the morphology and secretions of these glands with similar glands previously reported in other *Eurycea.*

Thirty-eight adult male cave salamanders (45-61 mm in snout-vent length [SVL], $\bar{x} = 55.9$ mm) were used in this study. The animals were taken from the Arkansas State University Museum of Zoology (ASUMZ) and from the personal collection of S. E. Trauth (SET). Specimens were collected from caves in the following counties of Arkansas: Fulton, Independence, Izard, and Stone. Collection dates were from December 1977 to July 1997. The visible glandular hump on the mid-dorsal region of the tail was measured and removed; in addition, an equivalent region of skin was excised from animals that did not possess these protuberances. The tissue samples were prepared for light microscopy using histological techniques outlined by Humason (1979); briefly, these steps were as follows: 1) dehydration in a graded series of ethanol, 2) clearing in xylene, and 3) embedding in paraffin. The tissue samples were oriented in the paraffin so that transverse or frontal sections could be obtained in a complete series. The tissue samples were cut at 8 µm using a rotary microtome. Four staining procedures were used and are as follows: hematoxylin-eosin (H&E) for general cytology, Pollak (Pollak) trichrome for connective tissues and mucosubstances, alcian blue 8GX at pH 2.8 for sulfated glycosaminoglycans, and periodic acid-Schiff's reagent (PAS) for general carbohydrates. These stains were alternately used on sequential groups of four slides. Glands were measured using a calibrated ocular micrometer and reported in µm; glandular volumes were derived using the formula for the volume of a cylinder.

Caudal courtship glands found in *Eurycea lucifuga* do not show a high degree of morphological variation. The glands tend to be either round or oblong in the pre-secretory stage (Fig. 1A), secretory stage (Fig. 2A, B, and C), and post-secretory stage (Fig 2D). The caudal courtships glands reside deep within the dermis and lie superficial to a layer of adipose tissue. The entire, elevated glandular hump ranged from 5.02 –11.9 mm in length ($\bar{x} = 7.69$ mm).

The epithelial lining of caudal courtship glands is columnar and variable in thickness ($\bar{x} = 60.2 \pm 5.2$ µm, range, 29.2 - 99.3, n = 30) in relation to secretory activity. These glands can be distinguished from other glands (namely, mucous and granular glands) by their size, staining properties, and secretions. Caudal courtship glands are usually greater in width ($\bar{x} = 168.2$ µm; range, 46.2 - 365.7, n = 130) and height ($\bar{x} = 190.1$ µm; range, 42.0 - 439.0; n = 130) than

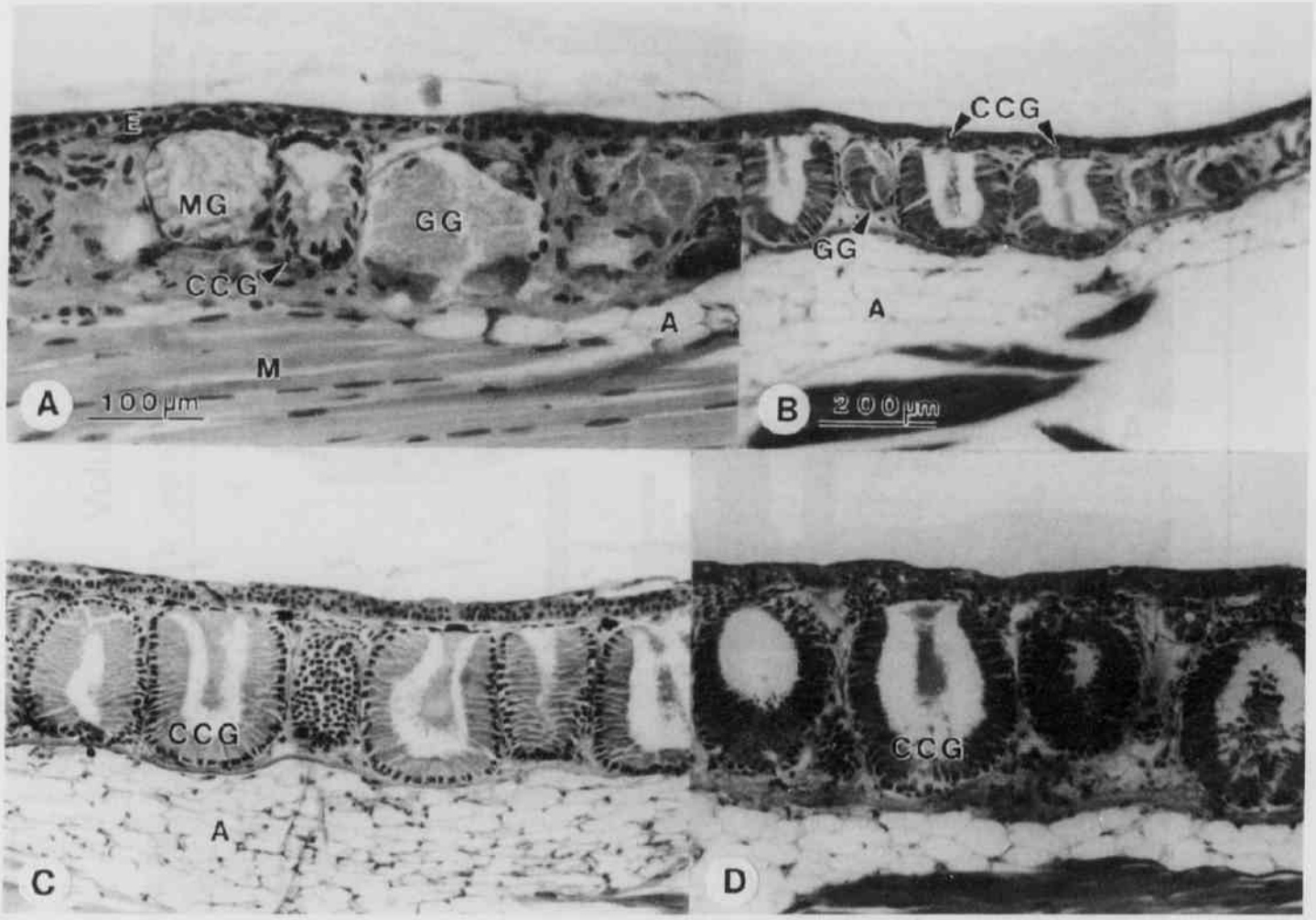

Fig. 1. Photomicrographs of sagittal sections through the mid-dorsal region of the tail directly above and posterior to the vent in *Eurycea lucifuga* illustrating the caudal courtship glands and their relationship to other epidermal glanda. A. Section of male skin (ASUMZ 20882) stained with H&E showing adipose tissue (A), mucous glands (MG), granular glands (GG), caudal courtship glands (CCG), a thin epidermis (E), and the dorsal musculature (M). Notice the relationship in size of the caudal courtship glands to the mucous and granular glands (specimen collected in February) before onset of the breeding season. B-D. Skin (ASUMZ 8147, 13966, SET 3841, respectively) stained with Pollak stain illustrating the relative increase in volume of the caudal courtship glands compared to other glands through the breeding season. Abbreviations the same as in A. Line in B the same for C and D.

either granular glands (width: $\bar{x}$ = 132.4 µm; range, 96.8 - 161.5; n = 19; height: $\bar{x}$ = 98.3 µm; range, 76.9 - 126.9, n = 19) or mucous glands (width: $\bar{x}$ = 64.3, µm; range, 38.5 - 107.7; n = 17; height: $\bar{x}$ = 48.2 µm; range, 19.2 - 103.8; n = 17). In addition, granular and mucous glands are mostly circular (Fig. 1A and B), whereas the caudal courtship glands are always barrel-like in shape (Fig. 1B and C).

Seasonal variation was observed in the secretory activity of caudal courtship glands of *Eurycea lucifuga* in Arkansas (Fig. 3). In specimens collected from October to early March caudal courtship glands were in a regressed state with most having little or no secretions. In contrast, caudal courtship glands examined from May-August possessed large amounts of secretory material and had greatly increased in size; the largest glandular volumes were observed in July.

The staining properties of the glandular secretions are similar to those that were reported in other *Eurycea* (Sever, 1989; Trauth et al., 1993). Glandular gland secretions are eosinophilic using H&E and Pollak, but they show no reac-

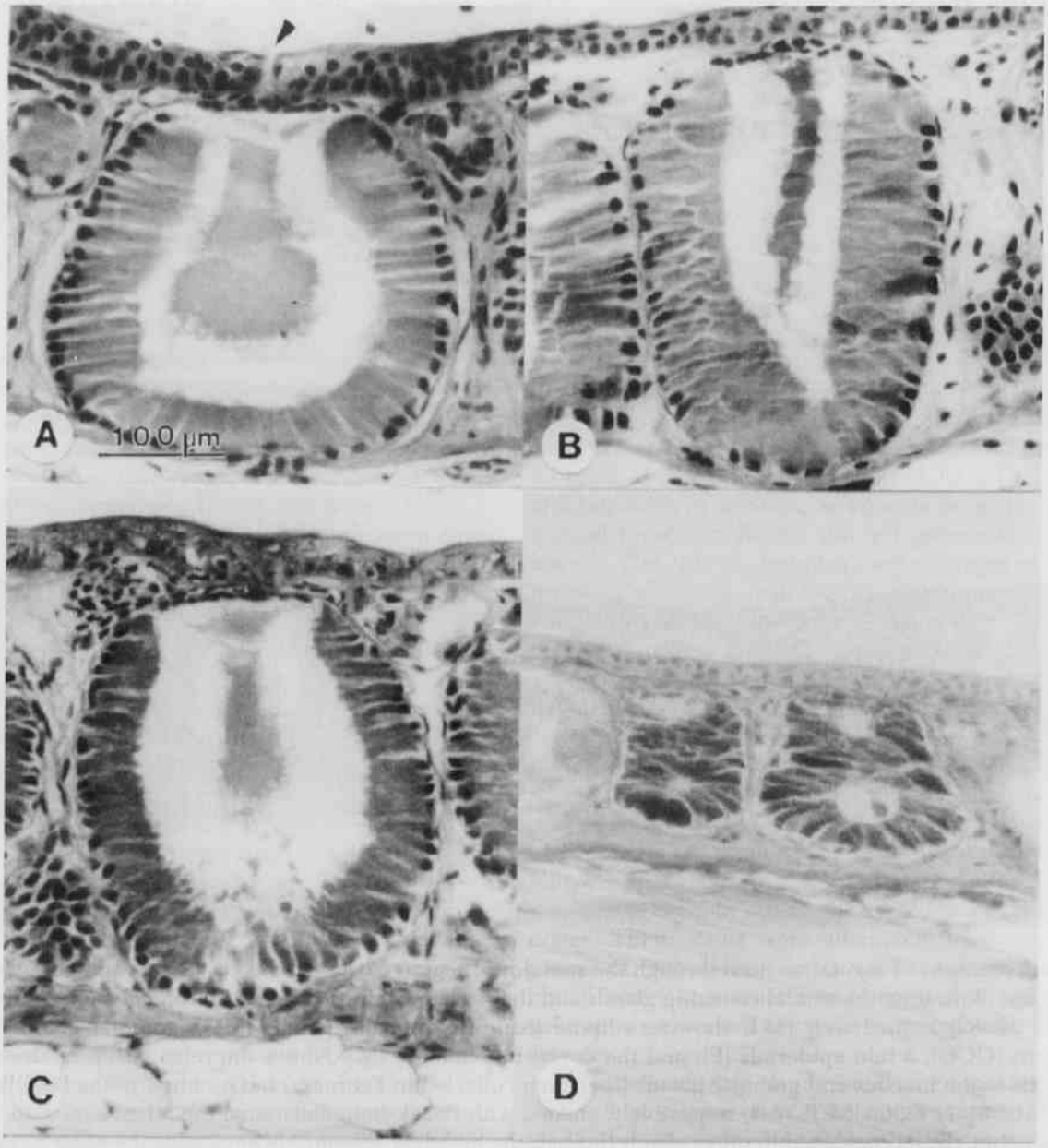

Fig. 2. A. Skin of male *Eurycea lucifuga* (ASUMZ 13966) stained with H&E illustrating the presence of a mucoprotein (eosinophilic) and columnar cells with basal nuclei; arrow indicates the duct opening. B-C. Skin (ASUMZ 13964; SET 3841, respectively) stained with Pollak stain illustrating the presence of mucosubstances. The epithelial lining of the caudal courtship gland appears light purple; the secretory column is a dark purple, except for portions that stain a dark brown to red. D. Skin of male (ASUMZ 14434) stained with PAS illustrating the regressed condition of the CCG following the breeding season (epithelial lining magenta in coloration). Line in A is the same for B-D.

tion with alcian blue, whereas the mucous glands contain a fibrous secretion that stains positive with alcian blue and basiophilic in H&E and Pollak. The staining characteristics of the caudal courtship glands indicate that a mucoprotein is involved as the secretory product (Sever, 1989; Trauth et al., 1993). In *E. lucifuga*, the secretion of the caudal courtship glands is PAS positive, alcian blue negative, and is eosinophilic using H&E. Staining with Pollak produced some mixed results. In many cases the secretion stained a light to dark blue, but in others the secretion was a dark

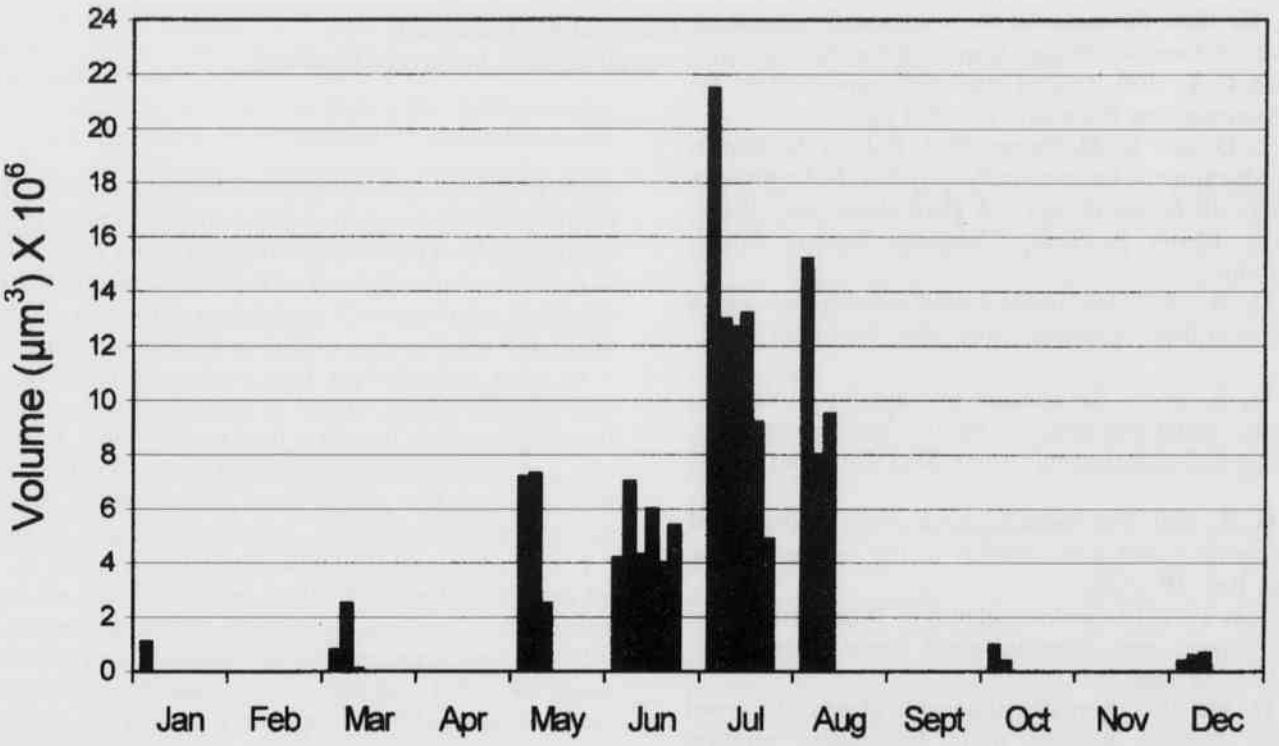

Fig. 3. Seasonal variation in average glandular volume of caudal courtship glands from 28 *Eurycea lucifuga*. Volumes represent values from five of the largest glands per specimen; the SVL's of specimens were as follows (linear order viewed in histogram): Jan - 58; Mar - 55, 57, 51; May - 58, 56, 55; Jun - 54, 58, 54, 58, 58, 54; Jul - 60, 54, 60, 58, 57, 61; Aug - 59, 55, 56; Oct - 61, 48; Dec - 53, 50, and 52.

brown or a shade of red.

The caudal courtship glands of *E. lucifuga* are similar in several respects to those of other species of *Eurycea* (*E. bislineata*; *E. cirrigera*; *E. junaluska*; *E. nana*; *E. wilderae*) as reported by Sever (1985, 1989) and in *E. multiplicata* (Noble, 1931). For instance, the round to barrel-like structure of these glands in the hypertrophied stage and the staining properties were consistent. The size of the caudal courtship glands in *E. lucifuga* is larger than those found in the other species of *Eurycea*, except for *E. longicauda melanopleura* (Trauth et al., 1993). *E. lucifuga* in the present study averaged around 8.2 mm greater in SVL compared to the *E. l. melanopleura* examined by Trauth et al. (1993).

In summary, cave salamanders (*Eurycea lucifuga*) were investigated for the presence of sexually dimorphic glands in the tail base. These multicellular, acinar, exocrine glands (caudal courtship glands) lie deep within the dermis in male *Eurycea* and produce a hypertrophied mid-dorsal area posterior to the vent. Caudal courtship glands can be distinguished from other glands (namely, mucous and granular) by morphology as well as the staining properties of the secretions. We found seasonal variation in the development of these glands; the glands exhibited their greatest volume in July at a time coinciding with ovarian enlargement in females and were least in volume during the winter months.

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